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New insights into human lysine degradation pathways with relevance to pyridoxine-dependent epilepsy due to antiquitin deficiency

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Abstract

Deficiency of antiquitin (ATQ), an enzyme involved in lysine degradation, is the major cause of vitamin B_6-dependent epilepsy. Accumulation of the potentially neurotoxic α-aminoadipic semialdehyde (AASA) may contribute to frequently associated developmental delay. AASA is formed by α-aminoadipic semialdehyde synthase (AASS) via the saccharopine pathway of lysine degradation, or, as has been postulated, by the pipecolic acid (PA) pathway, and then converted to α-aminoadipic acid by ATQ. The PA pathway has been considered to be the predominant pathway of lysine degradation in mammalian brain; however, this was refuted by recent studies in mouse. Consequently, inhibition of AASS was proposed as a potential new treatment option for ATQ deficiency. It is therefore of utmost importance to determine whether the saccharopine pathway is also predominant in human brain cells. The route of lysine degradation was analyzed by isotopic tracing studies in cultured human astrocytes, ReNcell CX human neuronal progenitor cells and human fibroblasts, and expression of enzymes of the two lysine degradation pathways was determined by Western blot. Lysine degradation was only detected through the saccharopine pathway in all cell types studied. The enrichment of ^{15}N-glutamate as a side product of AASA formation through AASS furthermore demonstrated activity of the saccharopine pathway. We provide first evidence that the saccharopine pathway is the major route of lysine degradation in cultured human brain cells. These results support inhibition of the saccharopine pathway as a new treatment option for ATQ deficiency.

KEYWORDS

α-Aminoadipic semialdehyde, antiquitin, lysine catabolism, pipecolic acid, saccharopine

Abbreviations: AAA, α-aminoadipic acid; AADAT/KAT2, kynurenine/alpha-aminoadipate aminotransferase; AASA, α-aminoadipic semialdehyde; AASS, α-aminoadipic semialdehyde synthase; ATQ, antiquitin; CRYM/KR, μ-crystallin/ketimine reductase; LC-MS/MS, liquid chromatography-mass spectrometry; MPE, molar percent enrichment; NPC, neural progenitor cells; P5CR, pyrroline-5-carboxylate reductase; P6C, Δ^1-piperideine-6-carboxylate; PA, pipecolic acid; PIPOX, pipecolic acid oxidase; PLP, pyridoxal 5′-phosphate.

Lisa M. Crowther and Déborah Mathis contributed equally to this work.

Barbara Plecko is the guarantor.

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1 | INTRODUCTION

Lysine degradation has been considered to have two distinct pathways, through saccharopine formation by ε-deamination, or through pipecolic acid (PA) formation by α-deamination or transamination (Figure 1), although the first enzyme of the PA pathway has not yet been identified.[1] Both proposed pathways lead to formation of Δ^1-piperideine-6-carboxylate (P6C) and its open form α-aminoadipic semialdehyde (AASA), which is then converted to α-aminoadipic acid (AAA) by the AASA dehydrogenase antiquitin (ATQ). Based on rat and mouse studies, it has long been believed that the PA pathway is predominant in the brain, while in other tissues the saccharopine pathway is more active.[1–5]

In ATQ deficiency, the most frequent cause of vitamin B_6/pyridoxine-dependent epilepsy, AASA and P6C accumulate, leading to inactivation of pyridoxal 5′-phosphate (PLP), the active vitamer of vitamin B_6. This occurs due to a condensation reaction between PLP and accumulated P6C.[6] Despite seizure control and reduction of AASA with high-dose vitamin B_6 (pyridoxine) treatment, around 70% of ATQ-deficient patients still suffer from developmental delay.[6] This could be due to AASA, which is highly reactive and possibly neurotoxic and which levels do not normalize.[7–11]

In the search for new treatment modalities, the lysine degradation pathway has gained new attention and the activity of the PA pathway has been brought into question by recent isotopic tracing studies. In fibroblasts from ATQ-deficient patients, PA derived only from the saccharopine pathway.[5] PA formation by the saccharopine pathway was demonstrated via conversion of P6C to PA by pyrroline-5-carboxylate reductase (P5CR),[12] in contrast to the previous assumption that PA is exclusively produced by the PA pathway. It was also recently shown that in mouse brain, liver, and kidney, lysine is not degraded to AAA by the PA pathway but only through the saccharopine pathway.[13,14] Development of inhibitors of α-aminoadipic semialdehyde synthase (AASS), the enzyme which converts lysine to saccharopine, was proposed as a possible new treatment strategy in patients with ATQ deficiency.[13,14] As any activity through the PA pathway could result in accumulation of AASA/P6C even if AASS is inhibited, it is important to determine whether the saccharopine pathway is also the major lysine degradation pathway in human brain, as the lysine degradation pathways appear to show species-specific activities.[15–19,33]

In this study, we used isotopic tracing studies to examine whether in human brain cells, AAA is formed by the PA pathway as has been deduced from previous studies, or by the saccharopine pathway as was recently demonstrated in mouse brain. It has been shown that ATQ is expressed in human astrocytes but not in neurons[20]; therefore, we examined the formation of AAA in astrocytes as well as in a human neural progenitor cell (NPC) line derived from the cortical region of human fetal brain (ReNcell CX NPCs), which we demonstrate express AASS and ATQ. In addition, we examined whether activity through the PA pathway might increase when the saccharopine pathway is impaired, by comparing AASS-deficient and ATQ-deficient human fibroblasts to control fibroblasts.

2 | METHODS

2.1 | Cell culture

Human fibroblasts (Life Technologies, Carlsbad, California, US), human astrocytes derived from human cortex (Sciencell Research Laboratories, Carlsbad, California, US), and ReNcell CX human NPCs (Millipore, Billerica, Massachusetts) were cultured according to the manufacturers' directions. Astrocytes were cultured for a maximum of 10 population doublings (four passages). ATQ-deficient fibroblasts were provided by B. Plecko and P. Mills (Institute of Child Health, University College London with Great Ormond Street Hospital for Children, National Health Service Trust, London, UK) and were previously characterized.[6] AASS-deficient fibroblasts were provided by J. Haeberle (Division of Metabolism, University Children's Hospital Zurich, Zurich, Switzerland) and have been previously described.[21] Deficiency of ATQ and AASS was shown by Western blot analysis (Figure S1), confirming the previously reported deleterious effects of mutations in these cells.[6,21]

2.1.1 | Tracing experiments with ^{15}N-labeled lysine

Cells were incubated for 24 hours in custom lysine-free media (Gibco, ThermoFisher Scientific, Waltham, MA USA) supplemented with L-[α^{15}N]-lysine (α^{15}N-Lys) (Cambridge Isotope Laboratories) or L-[ε^{15}N]-lysine (ε^{15}N-Lys) (Sigma-Aldrich, St. Louis, Missouri, USA) or unlabelled lysine to a final concentration of 0.44 g/L (Sigma-Aldrich).

Specifically, astrocytes were incubated in astrocyte media made from custom lysine-free Dubelcos modified Eagle

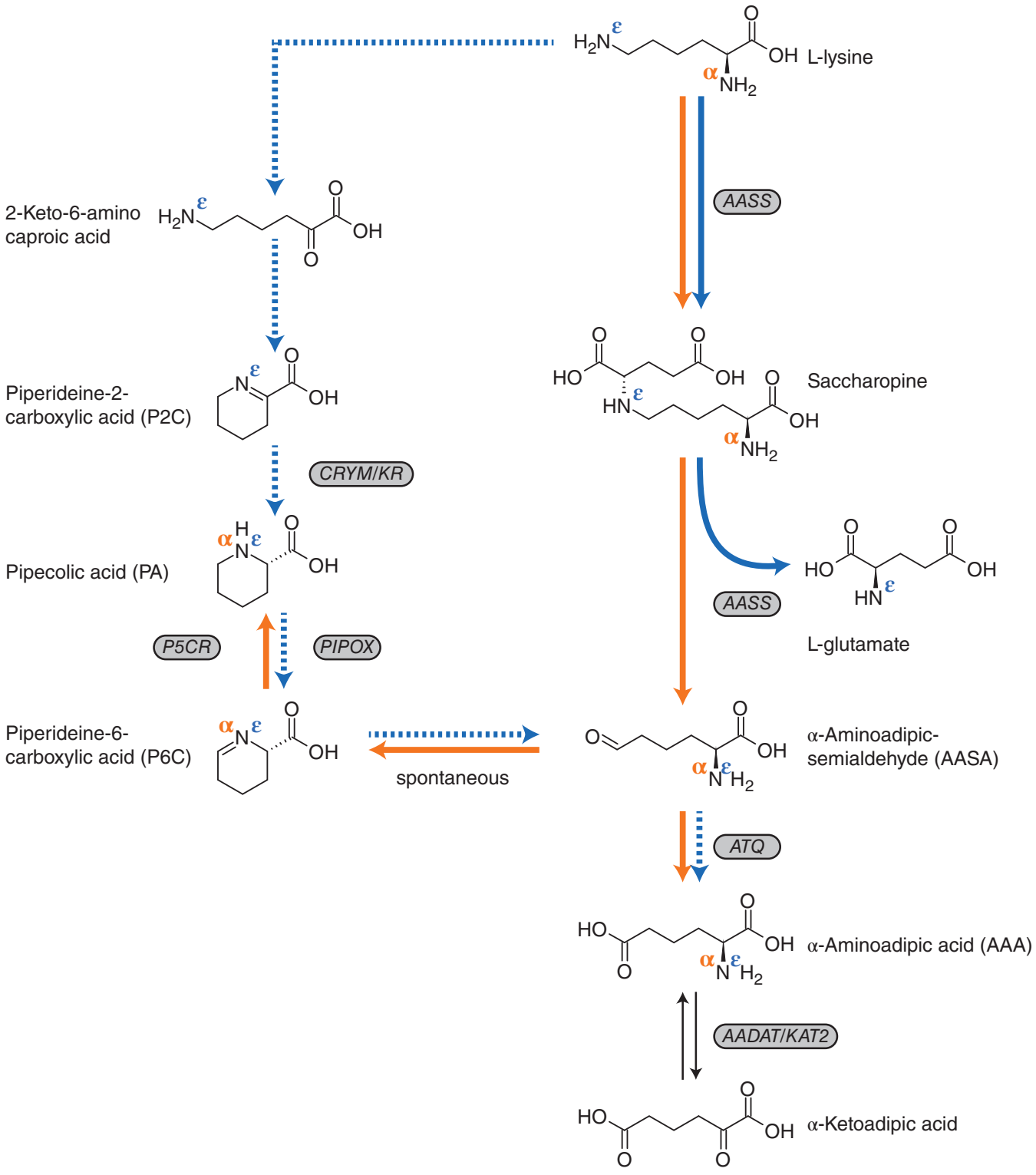

FIGURE 1 Lysine degradation pathway. The proposed pathways of lysine degradation through the PA pathway via α-deamination (dotted blue line) and the saccharopine pathway via ε-deamination (orange line), both leading to the formation of AASA/P6C and conversion of AASA to AAA. AAA, α-aminoadipic acid; AADAT/KAT2, kynurenine/alpha-aminoadipate aminotransferase; AASS, alpha-aminoadipic semialdehyde synthase; ATQ, antiquitin (α-aminoadipic semialdehyde dehydrogenase); CRYM/KR, μ-crystallin/ketimine reductase; P5CR, pyrroline-5-carboxylate reductase; P6C, Δ^1-piperideine-6-carboxylate; PA, pipecolic acid; PIPOX, pipecolic acid oxidase

medium (DMEM) supplemented with N2 supplement and one-shot Fetal bovine serum (FBS) (Gibco, ThermoFisher Scientific). ReNcell CX NPCs were incubated in custom lysine-free DMEM-F12 (3: 1) (Gibco, ThermoFisher Scientific) supplemented with B27 (2%), heparin (10 Units/ml, Sigma), fibroblast growth factor (FGF) (20 ng/mL), and epidermal growth factor (EGF) (20 ng/mL) for labeled lysine experiments, instead of ReNcell neural stem cell (NSC) media supplemented with FGF (20 ng/mL), and EGF (20 ng/mL), which was used for routine culture. Fibroblasts were incubated in custom lysine-free DMEM (Gibco, ThermoFisher Scientific) supplemented with 5% fetal calf serum (FCS) for labeled lysine experiments, rather than DMEM supplemented with 10% FCS as was used for routine culture.

2.1.2 | Cell harvesting and sample preparation for liquid chromatography-mass spectrometry

The volume of cell lysates analyzed was normalized by Hoescht assay as described previously[22] to account for differences in efficiency of cell transfer during harvesting (Supplementary Information). Cells were washed three times with ddH_20, then scraped using a rubber scraper into 50% methanol (Sigma-Aldrich). Cell extracts were sonicated for 15 sec then centrifuged at 14,500 *g* at 4 °C for 15 min. The supernatant was evaporated in a Concentrator Plus (Eppendorf) and resuspended in 10 µL of 50% methanol.

2.2 | Quantitative and qualitative analysis of PA, P6C, AASA, and AAA

The quantitative analysis of PA, P6C, AASA, AAA, and their respective labeled-ions (^{15}N-PA, ^{15}N-P6C, ^{15}N-AASA, and ^{15}N-AAA) in cell lysates and cell culture media was performed using a liquid chromatography-mass spectrometry (LC-MS/MS) system (Thermo Ultra High Performance Liquid Chromatography [UHPLC] Ultimate 3000XRS coupled to an SCIEX 5500 TripleQuad) with modifications of published protocols[5,23] (Supplementary Information). For all metabolites, the limit of detection and the limit of quantification were estimated at 0.004 µM (S/N = 3) and 0.013 µM (S/N = 10), respectively. Enrichment of ^{15}N-metabolites was expressed as molar percent enrichment (MPE), as described previously.[5] The quantification limit of MPE was calculated as <5%.

Qualitative analysis of lysine, PA, AAA, glutamate, and glutamine, as well as their isotopically labeled analogues was performed using an alternative UHPLC-MS/MS approach (Hydrophilic Interaction Liquid Chromatography [HILIC] chromatography utilizing a Thermo UHPLC Ultimate 3000XRS coupled to a QExactive high-resolution mass spectrometer, Supplementary Information).

2.3 | Western blot analysis

Western blots were performed following standardized procedures as described in the Supplementary Information. Primary antibodies and dilutions used were ATQ (1:50000; Abcam, Cambridge, UK), PA oxidase (PIPOX) (1:2000; Sigma-Aldrich, Buchs SG, Switzerland), AASS (1:2000; Abcam), and β-Actin (1:10000; Abcam). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from the legal guardians of the three patients who provided the fibroblasts for this study.

3 | RESULTS

3.1 | Expression of enzymes and activity of the PA and saccharopine pathway in cultured human cells

Western blot analysis showed that the enzymes ATQ, PIPOX, and AASS are expressed in human astrocytes, NPCs, and fibroblasts (Figure 2A).

In cultivated human astrocytes, NPCs, and fibroblasts, we detected lysine degradation through the saccharopine pathway and not the PA pathway, using isotopic tracing studies with L-[α^{15}N]-lysine (α^{15}N-Lys) and L-[ε^{15}N]-lysine (ε^{15}N-Lys). ^{15}N-labeled lysine degradation products were only detected in cells supplemented with α^{15}N-Lys and not in cells grown with ε^{15}N-Lys (Figure 2B).

^{15}N-labeled AAA was detected in astrocytes, NPCs, and fibroblasts only when the media was supplemented with α^{15}N-Lys, as shown by 40%-60% MPE of ^{15}N-labeled AAA (Figure 2B). The same results were also seen in human neural stem cells derived from human embryonic stem cells (hESCs) (Gibco, ThermoFisher Scientific) with 44% MPE of ^{15}N-labeled AAA in cells supplemented with α^{15}N-Lys (Figure S2). The 40%-60% MPE of ^{15}N-labeled AAA indicates that approximately half AAA was unlabelled, which is likely due to isotopic scrambling during the reversible, transaminase-induced conversion of AAA and α-ketoglutaric acid to α-ketoadipic acid and glutamate. In contrast, ^{15}N-labeled AAA was below the quantification limit (5% MPE) in cells grown in ε^{15}N-Lys, indicating that no PA pathway-derived AAA was detectable.

In astrocytes, low concentrations of AASA/P6C and PA were measured (close to the detection limit), derived exclusively from the saccharopine pathway (80%-100% MPE with α^{15}N-Lys, Figure S3). In NPCs, no AASA/P6C or PA was detected in cells incubated with lysine, and in fibroblasts, no AASA/P6C and low levels of PA were measured, only derived from the saccharopine pathway (50% MPE with α^{15}N-Lys,

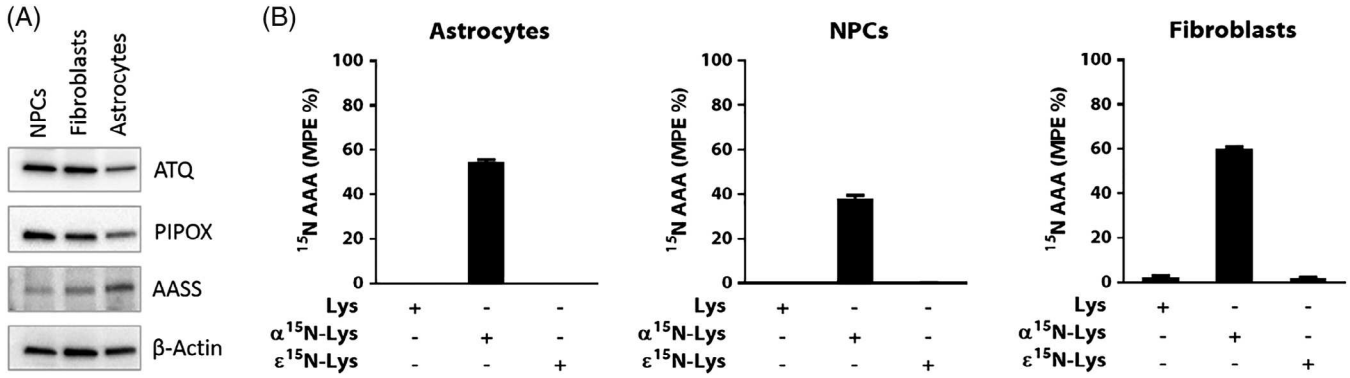

FIGURE 2 (A) Western blot showing expression of lysine degradation enzymes ATQ, PIPOX, and AASS in cultured human fibroblasts, astrocytes, and ReNcell CX NPCs. (B) ^{15}N-AAA in cell extracts. ^{15}N-AAA expressed as MPE (%) in extracts of fibroblasts, astrocytes, and ReNcell CX NPCs incubated in lysine-free media supplemented with Lys, α^{15}N-Lys, or ε^{15}N-Lys. Results are representative of at least two independent experiments. AAA, α-aminoadipic acid; AASS, α-aminoadipic semialdehyde synthase; ATQ, antiquitin; MPE, molar percent enrichment; NPC, neural progenitor cell; PIPOX, pipecolic acid oxidase

Figure S3). In cell culture media from astrocytes, NPCs and fibroblasts, labeled PA was detected, only from cells supplemented with α^{15}N-Lys and not ε^{15}N-Lys (Figure S4).

These results were confirmed by qualitative analysis of isotopically labeled PA and AAA in cell lysates using high-resolution MS (Figure S5), and activity of AASS was demonstrated by ^{15}N-glutamate enrichment studies. Increased ^{15}N-glutamate enrichment in cells incubated with ε-^{15}N-lysine (Figure 3) confirms conversion of saccharopine to AASA and glutamate as this involves transfer of ε-^{15}N to glutamate (Figure 1). This was detected in all cell types investigated in this study, although absolute levels of enrichment were shown to be rather low. This indicates that glutamate production by the saccharopine pathway contributes relatively little to overall glutamate production. In ATQ-deficient fibroblasts, lower ^{15}N-glutamate enrichment was detected for cells incubated with α^{15}N-Lys than in control fibroblasts (Figure 3), indicating that the labeled glutamate results from the transamination of AAA.

Interestingly, ^{15}N-glutamate enrichment in cells incubated in ε-^{15}N-lysine containing media was higher in ATQ-deficient fibroblasts than control fibroblasts suggesting increased AASS activity in ATQ-deficient compared to control fibroblasts (Figure 3). Glutamine, used as a negative control, did not exhibit enrichment beyond the natural abundance of 0.7% ^{15}N.

3.2 | Activity of the PA pathway in cultured fibroblasts with impaired saccharopine pathway

Furthermore, using isotopic tracing analysis, we examined whether lysine degradation through the PA pathway is increased when the first step of the saccharopine pathway is

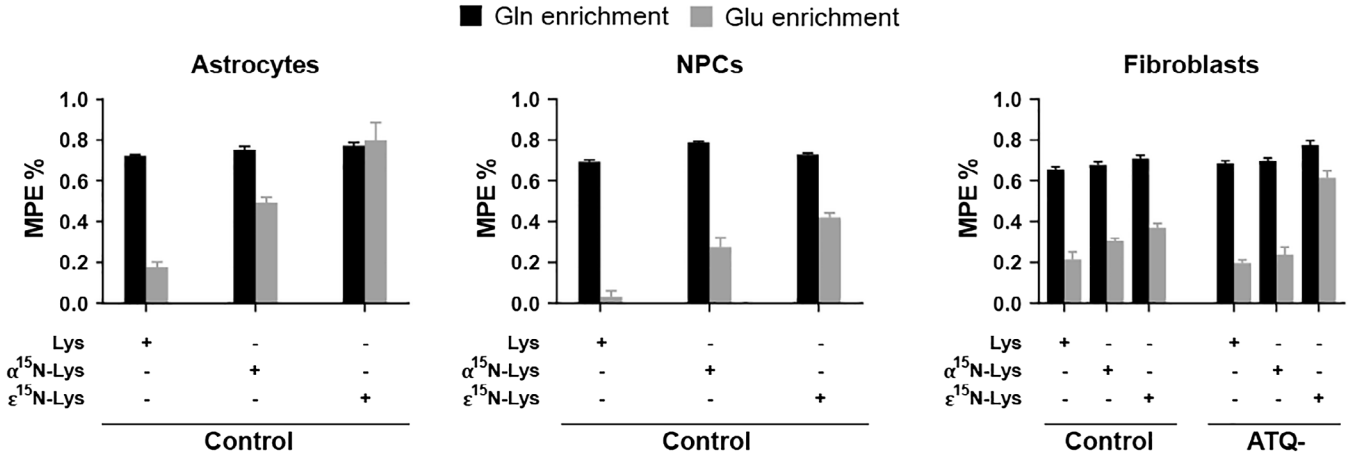

FIGURE 3 Enrichment of ^{15}N-labeled glutamine and glutamate in cell extracts. ^{15}N-labeled glutamine and glutamate expressed as MPE (%) in extracts of astrocytes, ReNcell CX NPCs and fibroblasts incubated in lysine-free media supplemented with Lys, α^{15}N-Lys, or ε^{15}N-Lys. The results depicted are representative of at least two independent experiments performed in one cell line per cell type; however, similar results were obtained with a second ATQ-deficient cell line. ATQ, antiquitin; Gln, glutamine; Glu, glutamate; MPE, molar percent enrichment; NPC, neural progenitor cell

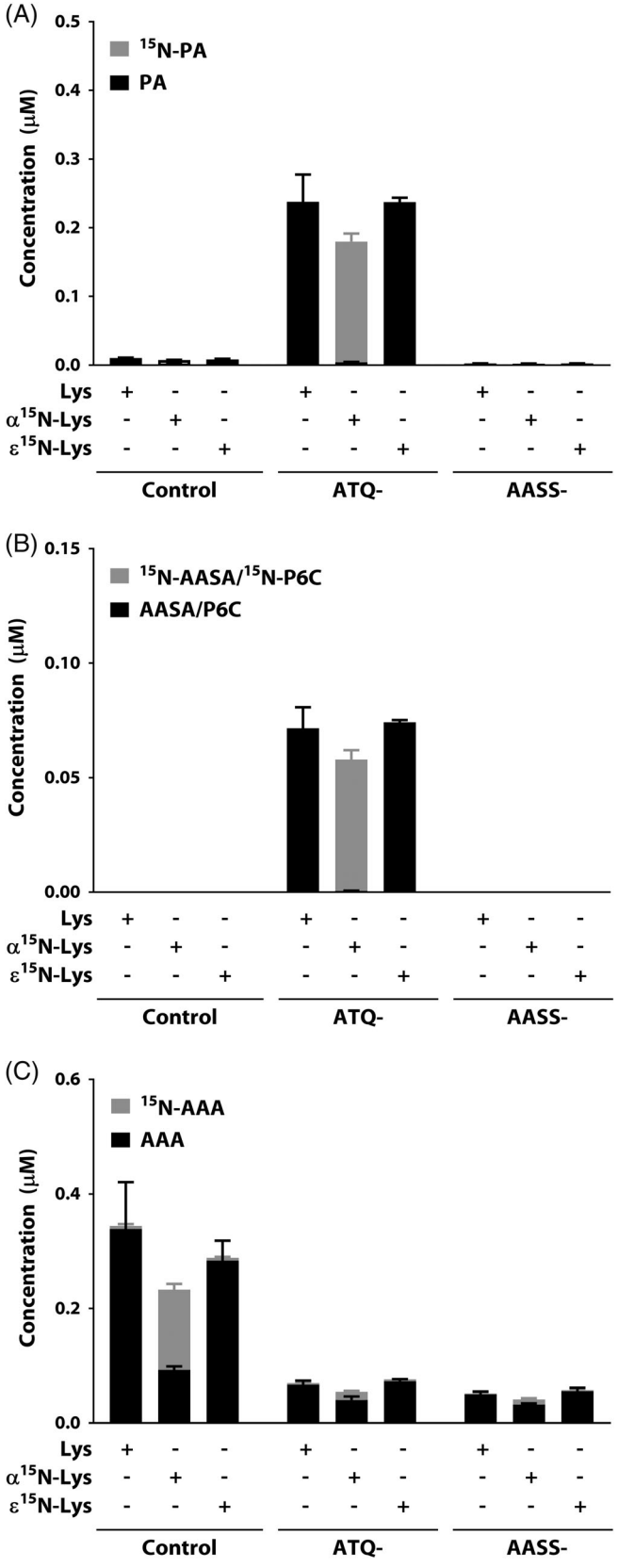

FIGURE 4 Legend on next coloumn.

impaired, using AASS-deficient fibroblasts, compared to ATQ-deficient and control fibroblasts.

AASA/P6C and PA were not detectable in AASS-deficient cells, whereas in ATQ-deficient cells, AASA/P6C and PA concentrations were increased compared to control fibroblasts. AAA concentrations were not higher in AASS-deficient fibroblasts than background levels detected in ATQ-deficient fibroblasts (Figure 4). Furthermore, in ATQ-deficient fibroblasts, ^{15}N-AASA/P6C and ^{15}N-PA were produced only in cells supplemented with α^{15}N-Lys therefore derived exclusively from the saccharopine pathway (Figure 4A and B). Furthermore and as expected, no enrichment of ^{15}N-glutamate to unlabeled glutamate was observed in AASS-deficient fibroblasts (Figure S6). These results illustrate that in fibroblasts, degradation of lysine does not occur via the PA pathway even when the saccharopine pathway is impaired.

4 | DISCUSSION

While it has long been reported that the PA pathway is the predominant route of lysine degradation in the brain, a reappraisal of the importance of the saccharopine pathway in brain is warranted. We examined the route of production of AAA in human astrocytes and in a human NPC line derived from the cortical region of human fetal brain (ReNcell CX NPCs), which express AASS, PIPOX, and ATQ, but not in neurons, as it has been shown that in human brain, neurons do not express ATQ.[20] Conclusions of our study are based on the widely adopted hypothesis that the first step of the PA pathway occurs via lysine α-transamination or deamination.

Data generated within our study revealed that conversion of lysine to AAA is only detectable through the saccharopine pathway in cultured human astrocytes and human NPCs, which is in line with the recent report that formation of AAA in lysine degradation occurs by the saccharopine pathway in mouse brain.[13,14] Our isotopic tracing studies showed conservation of the α-^{15}N label in all lysine

FIGURE 4 ^{15}N-labeled and unlabelled lysine catabolites in fibroblast lysates. Concentrations of PA (A), AASA/P6C (B), and AAA (C) in control, ATQ-deficient, and AASS-deficient fibroblasts incubated in lysine-free media supplemented with 5% FCS and Lys, α^{15}N-Lys or ε^{15}N-Lys, as indicated. For all metabolites, the limit of detection and the limit of quantification were estimated at 0.004 μM (S/N = 3) and 0.013 μM (S/N = 10), respectively. The results depicted are representative of at least two independent experiments performed in one cell line per cell type; however, similar results were obtained with a second ATQ-deficient cell line. AAA, α-aminoadipic acid; AASA, α-aminoadipic semialdehyde; AASS, α-aminoadipic semialdehyde synthase; ATQ, antiquitin; PA, pipecolic acid

degradation products detected. Glutamate enrichment studies also demonstrated transfer of the ε-^{15}N to glutamate, indicating conversion of saccharopine to AASA and glutamate as previously shown in mouse cerebellar and cerebral slices.[24] As such we confirmed ε-deamination or transamination of lysine (the saccharopine pathway) as an active route of lysine degradation in human astrocytes and NPCs.

In view of our findings, previous research results deserve special consideration. In 1954, Rothstein and Miller reported that in rat, PA and AAA were derived only from the PA pathway and not through the saccharopine pathway of lysine degradation.[25] This was deduced from analysis of urine after IP injection of α^{15}N- or ε^{15}N-DL-lysine and was based on the assumption that L-PA derived from L-lysine; however, it was later shown that L-PA is metabolized not only from L-lysine but also D-lysine[3,16] by D-amino acid oxidase.[19] Therefore, the formation of AAA by the PA pathway in this study cannot be attributed only to L-lysine degradation and may have resulted from degradation of D-lysine.

It was subsequently reported that the saccharopine pathway of degradation is active in tissues such as liver and kidney,[2–4,26–28] but that the PA pathway is the predominant pathway in brain. The importance attributed to the PA pathway in brain was deduced from the inability to detect saccharopine in lysine degradation in rat brain in early studies,[3] low expression of AASS in mammalian brain compared to liver, heart, and kidney,[3,4,18,27,29] and the decrease in AASS enzyme activity in the first week of postnatal life.[28] Saccharopine has since been detected in several studies in mouse brain,[13,14,18] and it has been proposed that the difficulty in detecting saccharopine in early studies may have been due to time of sampling,[13,14] as well as the lower efficiency of lysine conversion to saccharopine by saccharopine dehydrogenase than the conversion of saccharopine to AASA by lysine-oxoglutarate reductase, resulting in low concentrations of saccharopine.[30] In mammals, the bifunctional enzyme AASS possesses activities of both these enzymes.[31,32]

Furthermore, the detection of PA as a lysine degradation product in brain was considered evidence of activity through the PA pathway of lysine degradation.[3] However, a recent study in ATQ-deficient fibroblasts showed that PA can be produced by the saccharopine pathway,[5] and this was also shown to occur in mouse brain, although more PA was produced by the PA pathway as shown by higher ^{15}N-PA enrichment in brain from ε-^{15}N-lysine and α-^{15}N-lysine treated mice.[13,14,18] Our data in human astrocytes support and extend previous findings in human fibroblasts showing that PA is produced via the saccharopine pathway, by P6C conversion to PA.[5] This indicates species differences in the conversion of L-lysine to PA by the PA pathway, as this was detected in mouse but not in human cells.

We also showed that in fibroblasts with impaired saccharopine pathway activity due to AASS deficiency, there was still no detectable production of PA or AAA by the PA pathway.

Preliminary findings suggest that AASS activity in ATQ-deficient fibroblasts could be increased compared to control fibroblasts, as shown by glutamate enrichment studies (Figure 3). This may indicate feedback inhibition by AAA or a downstream metabolite and increased rate of lysine degradation through the saccharopine pathway in ATQ deficiency. Future studies investigating ATQ deficiency in human brain cells would be important to extend these findings to brain, as any increase in AASS activity due to ATQ deficiency would further support the potential benefits of inhibiting AASS (and therefore preventing AASA accumulation) in ATQ deficiency.

5 | CONCLUSIONS

Our study provides the first evidence that the saccharopine pathway is the main route for lysine degradation to AAA in human astrocytes and a human NPC line derived from the cortical region of human fetal brain (ReNcell CX NPCs), and extends previous data in human fibroblasts. Results of our experiments in addition to previous studies indicate species differences in the activity of steps of the lysine degradation pathway. In mouse, PA is produced by both α- and ε-deamination, while in all human cells investigated to date, PA is only produced by ε-deamination. Recognition of the relevance of the saccharopine pathway of lysine degradation in human brain tissue may have been delayed due to differing activity of each step of the PA and saccharopine pathways depending on species, tissue as well as age-dependent effects.[28] While future studies using astrocyte-neuronal co-cultures may provide further information on possible shuttling of lysine degradation products between neurons and astrocytes, our findings indicate the importance of the saccharopine pathway in human brain cells, supporting the idea that inhibition of the saccharopine pathway could offer new treatment options for ATQ deficiency.

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providing the ATQ- and AASS-deficient fibroblasts, respectively.

CONFLICT OF INTEREST

L.M.C. has received travel reimbursement from Nutricia in 2017. D.M. and M.P. declare that they have no conflict of interest. B.P. has received honoraria from Alexion in 2017 and travel reimbursement from Nutricia in 2018.

AUTHOR CONTRIBUTIONS

L.M.C. drafted the study design, conducted all cell culture experiments and Western blot analysis, interpreted the results, drafted the manuscript, and contributed to drafting figures. D.M. established the LC-MS method for analysis of the isotopic tracing studies, ran and interpreted all assays and results, drafted the figures, and contributed to drafting the manuscript. M.P. drafted several figures, gave input to data interpretation, and contributed to drafting the manuscript. B.P. gave substantial input to the study design, data interpretation, and to the manuscript.

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REFERENCES

1. Hallen A, Jamie JF, Cooper AJ. Lysine metabolism in mammalian brain: an update on the importance of recent discoveries. *Amino Acids*. 2013;45:1249-1272.
2. Chang YE. Lysine metabolism in the rat brain: the pipecolic acid-forming pathway. *J Neurochem*. 1978;30:347-354.
3. Chang YF. Pipecolic acid pathway: the major lysine metabolic route in the rat brain. *Biochem Biophys Res Commun*. 1976;69:174-180.
4. Sauer SW, Opp S, Hoffmann GF, Koeller DM, Okun JG, Kolker S. Therapeutic modulation of cerebral L-lysine metabolism in a mouse model for glutaric aciduria type I. *Brain*. 2011;134:157-170.
5. Struys EA, Jakobs C. Metabolism of lysine in alpha-aminoadipic semialdehyde dehydrogenase-deficient fibroblasts: evidence for an alternative pathway of pipecolic acid formation. *FEBS Lett*. 2010;584:181-186.
6. Mills PB, Footitt EJ, Mills KA, et al. Genotypic and phenotypic spectrum of pyridoxine-dependent epilepsy (ALDH7A1 deficiency). *Brain*. 2010;133:2148-2159.
7. Coughlin CR 2nd, van Karnebeek CD, Al-Hertani W, et al. Triple therapy with pyridoxine, arginine supplementation and dietary lysine restriction in pyridoxine-dependent epilepsy: neurodevelopmental outcome. *Mol Genet Metab*. 2015;116:35-43.
8. Mercimek-Mahmutoglu S, Cordeiro D, Cruz V, et al. Novel therapy for pyridoxine dependent epilepsy due to ALDH7A1 genetic defect: L-arginine supplementation alternative to lysine-restricted diet. *Eur J Paediatr Neurol*. 2014a;18:741-746.
9. Mercimek-Mahmutoglu S, Corderio D, Nagy L, et al. Lysine-restricted diet and mild cerebral serotonin deficiency in a patient with pyridoxine-dependent epilepsy caused by ALDH7A1 genetic defect. *Mol Genet Metab Rep*. 2014b;1:124-128.
10. Plecko B, Paul K, Paschke E, et al. Biochemical and molecular characterization of 18 patients with pyridoxine-dependent epilepsy and mutations of the antiquitin (ALDH7A1) gene. *Hum Mutat*. 2007;28:19-26.
11. van Karnebeek CD, Hartmann H, Jaggumantri S, et al. Lysine restricted diet for pyridoxine-dependent epilepsy: first evidence and future trials. *Mol Genet Metab*. 2012;107:335-344.
12. Struys EA, Jansen EE, Salomons GS. Human pyrroline-5-carboxylate reductase (PYCR1) acts on Delta(1)-piperideine-6-carboxylate generating L-pipecolic acid. *J Inherit Metab Dis*. 2014;37:327-332.
13. Pena IA, Marques LA, Laranjeira AB, Yunes JA, Eberlin MN, Arruda P. Simultaneous detection of lysine metabolites by a single LC-MS/MS method: monitoring lysine degradation in mouse plasma. *Springerplus*. 2016a;5:172.
14. Pena IA, Marques LA, Laranjeira AB, et al. Mouse lysine catabolism to aminoadipate occurs primarily through the saccharopine pathway; implications for pyridoxine dependent epilepsy (PDE). *Biochim Biophys Acta Mol Basis Dis*. 2016b;1863(1):121-128.
15. Ghadimi H, Chou WS, Kesner L. Biosynthesis of saccharopine and pipecolic acid from L- and DL- 14 C-lysine by human and dog liver in vitro. *Biochem Med*. 1971;5:56-66.
16. Grove J, Henderson LM. The metabolism of D- and L-lysine in the intact rat, perfused liver and liver mitochondria. *Biochim Biophys Acta*. 1968;165:113-120.
17. Mihalik SJ, Rhead WJ. L-pipecolic acid oxidation in the rabbit and cynomolgus monkey. Evidence for differing organellar locations and cofactor requirements in each species. *J Biol Chem*. 1989;264:2509-2517.
18. Posset R, Opp S, Struys EA, et al. Understanding cerebral L-lysine metabolism: the role of L-pipecolate metabolism in Gcdh-deficient mice as a model for glutaric aciduria type I. *J Inherit Metab Dis*. 2015;38:265-272.
19. Zaar K, Angermuller S, Volkl A, Fahimi HD. Pipecolic acid is oxidized by renal and hepatic peroxisomes. Implications for Zellweger's cerebro-hepato-renal syndrome (CHRS). *Exp Cell Res*. 1986;164:267-271.
20. Jansen LA, Hevner RF, Roden WH, Hahn SH, Jung S, Gospe SM Jr. Glial localization of antiquitin: implications for pyridoxine-dependent epilepsy. *Ann Neurol*. 2014;75:22-32.
21. Houten SM, Te Brinke H, Denis S, et al. Genetic basis of hyperlysinemia. *Orphanet J Rare Dis*. 2013;8:57.
22. Muschet C, Moller G, Prehn C, de Angelis MH, Adamski J, Tokarz J. Removing the bottlenecks of cell culture metabolomics: fast normalization procedure, correlation of metabolites to cell number, and impact of the cell harvesting method. *Metabolomics*. 2016;12:151.
23. Mills PB, Struys E, Jakobs C, et al. Mutations in antiquitin in individuals with pyridoxine-dependent seizures. *Nat Med*. 2006;12:307-309.
24. Papes F, Surpili MJ, Langone F, Trigo JR, Arruda P. The essential amino acid lysine acts as precursor of glutamate in the mammalian central nervous system. *FEBS Lett*. 2001;488:34-38.

25. Rothstein M, Miller LL. The conversion of lysine to pipecolic acid in the rat. *J Biol Chem*. 1954;211:851-858.
26. Hutzler J, Dancis J. Conversion of lysine to saccharopine by human tissues. *Biochim Biophys Acta*. 1968;158:62-69.
27. Hutzler J, Dancis J. Lysine-ketoglutarate reductase in human tissues. *Biochim Biophys Acta*. 1975;377:42-51.
28. Rao VV, Pan X, Chang YF. Developmental changes of L-lysine-ketoglutarate reductase in rat brain and liver. *Comp Biochem Physiol B*. 1992;103:221-224.
29. Papes F, Kemper EL, Cord-Neto G, Langone F, Arruda P. Lysine degradation through the saccharopine pathway in mammals: involvement of both bifunctional and monofunctional lysine-degrading enzymes in mouse. *Biochem J*. 1999;344(Pt 2):555-563.
30. Fellows FC, Lewis MH. Lysine metabolism in mammals. *Biochem J*. 1973;136:329-334.
31. Markovitz PJ, Chuang DT, Cox RP. Familial hyperlysinemias. Purification and characterization of the bifunctional aminoadipic semialdehyde synthase with lysine-ketoglutarate reductase and saccharopine dehydrogenase activities. *J Biol Chem*. 1984;259:11643-11646.
32. Sacksteder KA, Biery BJ, Morrell JC, et al. Identification of the alpha-aminoadipic semialdehyde synthase gene, which is defective in familial hyperlysinemia. *Am J Hum Genet*. 2000;66:1736-1743.
33. Dancis J, Hutzler J. Comparative rates of metabolism of pipecolic acid in several animal species. *Comp Biochem Physiol B*. 1982;73:1011-1012.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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